A Project Report

on

Evaluation of biomass production of *Spirulina maxima* on different reported media and small scale Spirulina culture in plastic bowls and concrete tank

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Funded By: **IDRS-BFRI, Shrimp Research Institute, Bagerhat, Khulna**

Introduction

Spirulina is a cyanobacterium, which acquired the ability for photosynthesis before anyother organism and is considered to be the ancestor from which the higher plants evolved. The protein is an essential component of diet. The greatest single problem in the world today is Global food protein shortage. With the current system of production, agriculture cannot be relied upon to feed an ever increasing world population. Hence, there is an urgent need to find other protein sources. The best potential is seen in microbial protein or single cell protein (SCP), a new source of protein independent of agriculture. The dried cells of microorganisms such as bacteria, fungi, yeasts and algae that are grown in large scale culture systems as proteins, for human or animal consumption are collectively known as single cell protein. SCP are characterized by; fast growth rate; high protein content (43-85%) compared to field crops; require less water, land and independent of climate; grow on wastewater; can be genetically modified for desirable characters such as amino acid composition and temperature tolerance tolerance. Spirulina are multicellular and filamentous blue green algae that gained popularity among biologist and technologist. Spirulina was rediscovered in the mid-1960s. A French botanist, Jean Leonard, during his expedition to Africa, described a blue-green cake sold in the food market of Fort Lamy, Chad. Called in the local dialect as Dihe, this cake was consumed by the Kanembu tribe living along the alkaline lakes of Chad and Niger. In the 16th century, when Spanish invaders conquered Mexico, they discovered that the Aztecs living in the valley of Mexico, in the capital Tenochtitlan, were using a bluegreencake known as tecuitlatl as food. This was obtained from the lake in a similar way to Dihe. Although no evidence is available on the exact composition of this food, it is clear that it contained the blue-green alga *Spirulina maxima* which is the dominant species in those waters.Spirulina has found wide applications in the areas of agriculture, food, pharmaceuticals, perfumeries, medicine and science. Nowadays, this organism is used as a food supplement and is marketed in the form of pills, capsules and powder or incorporated into various types of food like cakes, biscuits, noodles and health drinks, etc. Various countries are developing strategic programs for the production and use of Spirulina. This present study is conducted to investigate about the biomass production of *Spirulina maxima* on different reported media and small scale Spirulina culture in plastic bowls and concrete tank.

General characteristics of spirulina

Morphology

Spirulina is symbiotic, multi-cellular and filamentous blue green microalgae with symbiotic bacteria that fix nitrogen from air. Spirulina can be rod shape or spiral shape. Their main photosynthetic pigment is phycocyanin, which is blue in color. These bacteria also contain chlorophyll a and carotenoids. Some contains the pigment phycoythrin, giving the bacteria a red or pink color. Spirulina are photosynthetic and therefore autotrophic. Spirulina reproduce by binary fission. The helical shape of the filaments (or trichomes) is characteristic of the genus and is maintained only in aliquid environment or culture medium. The presence of gas-filled vacuoles in the cells, together with thehelical shape of the filaments, result in floating mats. The trachomas have a length of 50 to 500 μm and awidth of 3 to 4 μm. However, Cyanobacteria have a cell wall similar to that of Gram-negative bacteria:they contain peptidoglucan, a lysozyme-sensitive heteropolymer that confers shape and osmotic protection to the cell, in addition to other material not sensitive to lysozyme.

Taxonomy

In 1827, P.J. Turpin isolated spirulina from a freshwater sample (Ciferri, 1983). In 1844, Wittrock and Nordstedt reported the presence near the city of Montevideo of helical, septal and green-blue microalgae named *Spirulinajenneri*f. *maxima*. The worldwide investigation on microalgae has been carried out under the name of"spirulina"; this common designation between scientists and consumers has proved difficult to change.The microalgae under discussion belong to the genus *Arthrospira*, but it will probably be called *Spirulina*for some time. For the purpose of this report we refer to both collectively as "spirulina".

Biochemical Composition

Proteins

Spirulina contains unusually high amounts of protein, between 55 and 70 percent by dry weight,depending upon the source (Phang*et al*., 2000). It is a complete protein, containing all essential aminoacids, though with reduced amounts of methionine, cystine, and lysine, as compared to standard proteinssuch as that from meat, eggs, or milk; it is, however, superior to all standard plant protein, such as that from legumes.

Essential fatty acids

Spirulina has a high amount of polyunsaturated fatty acids (PUFAs), 1.5–2.0 percentof 5–6 percent total lipid. In particular spirulina is rich in γ-linolenic acid (36 percent of total PUFAs), andalso provides γ-linolenic acid (ALA), linoleic acid (LA, 36 percent of total), stearidonic acid (SDA),eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA).

Vitamins

Spirulina contains vitamin B1 (thiamine), B2 (riboflavin), B3 (nicotinamide), B6 (pyridoxine), B9 (folic acid), B12 (cyanocobalamin), vitamin C, vitamin D and vitamin E.

Minerals

All the essential minerals are available in spirulina which contributes about 7 percent (average range 2.76–3.00 percent of total weight) under laboratory conditions. But in commercial spirulina production, minerals contribute about 7 percent. It bioaccumulates minerals when grown in different media, at different temperatures, pH, salinity, etc.Spirulina is a rich source of potassium, and also contains calcium, chromium, copper, iron,magnesium, manganese, phosphorus, selenium, sodium and zinc.

β-carotene and vitamins

The β-carotene, B-group vitamin, vitamin E, iron, potassium and chlorophyll available in the spirulina can promote the metabolism of carbohydrate, fats, protein, alcohol, and the reproduction of skin, muscle and mucosa. Spirulina contains large amounts of natural β-carotene and this β-carotene is converted into vitamin A. According to the findings of the National Cancer Institute, United States of America, an intake of 6.0 mg β-carotene daily may be effective in minimizing the risk of cancer. If anybody takes 4.0 g spirulina daily, that is sufficient to get 6 mg β-carotene. At the same time, sufficient amount of B-group vitamins, iron and calcium will be obtained. However, these nutrients obtained from 4.0 g of spirulina are equivalent to or more than those obtained by eating more than 100 g of terrestrial bright-coloured vegetables.

Photosynthetic pigments:

Spirulina contains many pigments including chlorophyll a, xanthophyll, betacarotene,echinenone, myxoxanthophyll, zeaxanthin, canthaxanthin, diatoxanthin, 3-hydroxyechinenone, beta-cryptoxanthin, oscillaxanthin, plus the phycobiliproteins c-phycocyanin and allophycocyanin.Detailed biochemical composition analyses have been conducted of spirulina grown either underlaboratory conditions, collected in natural condition or

in mass culture system using different agro-industrialwaste effluent. This was found to vary in response to the salinity of the growing medium Vonshak*et al*. (1996) reported that salt-adapted cells had a modified biochemical composition with areduced protein and chlorophyll content, and increased carbohydrate content. However the followingprovides a review of the literature on the broad composition of spirulina.

Proximate composition

Spirulina has high quality protein content (59–65 percent), which is more than other commonly usedplant sources such as dry soybeans (35 percent), peanuts (25 percent) or grains (8–10 percent). A specialvalue of spirulina is that it is readily digested due to the absence of cellulose in its cell walls (as it is thecase for eukaryotic green microalgae such as *Chlorella*, after 18 hours more than 85 percent of its protein is digested and assimilated (Sasson, 1997). Thecomposition of commercial spirulina powder is 60 percent protein, 20 percent carbohydrate, 5 percentfats, 7 percent minerals, and 3–6 percent moisture, making it a low-fat, low calorie, cholesterol-free source of protein.

Table 1: Various proximate analysis results of spirulina (% dry matter)

Component	**FOI, France**	**SAC,Thailand**	**IPGSR, Malaysia**	**BAU,Bangladesh**	**JUST**
Crude protein	65	55–70	61	60	56.05
Soluble carbohydrate	19	--	14	--	--
Crude lipid	4	5-7	6	7	--
Crude fiber	3	5-7	--	--	--
Ash	3	3-6	9	11	--
Moisture	--	4-6	6	9	--
Nitrogen free extract (NFE)	--	15-20	4	17	--

Cultivation and production of Spirulina

Methodology

In JSTU Fisheries and Marine Bioscience Laboratory we use Revised media-6, OFERR media, CFTRI media, Zarruk media, Rao’s media and JUST media (Self media) for culture. For Spirulina culture there we need some essential element present in the media. Such as Nine major environmental factors influence the productivity of spirulina: luminosity (photo-period 12/12,4

luxes), temperature (30 °C), inoculation size, stirring speed, dissolved solids (10–60 g/liter), pH (8.5–10.5), water quality, and macro and micronutrient presence (C, N, P, K, S, Mg, Na, Cl, Ca and Fe, Zn, Cu,Ni, Co, Se) (Ciferri, 1983; Ayala, 1998).

Room Preparation

We built a bamboo made room outside the academic building. But due to cyclone Mohasen the building was crashed and then we shift our culture media into the laboratory.

Figure 1: House Crashed by cyclone Mohasen.

Media Preparation

The culture of spirulina is practiced in different media, especially inorganic and decomposed organic nutrients. Different types of spirulina were cultured to evaluate growth and biochemistry under similar controlled conditions (Bhattacharya and Shivaprakash, 2005). *Spirulina maxima* showed highest growth rate, biomass, pigment concentration and low intracellular phenolics. The results indicate that *Spirulina maxima* reached highest growth in shortest doubling time and the importance of strain selection for large-scalecultivation. Sanchez-Luna *et al*. (2004) found that the intermittent addition of urea in the autotrophic culture of *Spirulina maxima* yielded similar results to those obtained by the continuous feeding.

In our culture period we used to culture six media as Revised media-6, OFERR media, CFTRI media, Zarruk media, Rao's media and JUST media (Self media)

The combination of Revise media-6 as,

Ingredients	g/l
Calcium Chloride	0.04

Sodium Chloride	0.50
Triple Super Phosphate	1.25
Muriate of Potash	0.89
Magnesium Sulphate	0.15
Sodium bicarbonate	8.0
Sodium Nitrate	2.5

JUST (self media) is form by following combination

Ingredients	g/l
Calcium Chloride	0.04
Sodium Chloride	0.50
Triple Super Phosphate	1.25
Potassium Nitrate	1
Magnesium Sulphate	0.15
Sodium bicarbonate	8.0
Sodium Nitrate	1.25
Cooking Soda	1.5

The combination of OFERR media as

Ingredients	g/l
Calcium Chloride	0.04
Sodium Chloride	0.50
Triple Super Phosphate	1.25
Potassium Nitrate	1
Magnesium Sulphate	0.15
Sodium bicarbonate	8.0
Sodium Nitrate	1.25
Cooking Soda	1.5

The combination of Zarruk media as

Ingredients	g/l
Calcium Chloride	0.04
Sodium Chloride	0.50

Triple Super Phosphate	1.25
Potassium Nitrate	1
Magnesium Sulphate	0.15
Sodium bicarbonate	8.0
Sodium Nitrate	1.25
Cooking Soda	1.5

The combination of CFTRI media as

Ingredients	g/l
Calcium Chloride	0.04
Sodium Chloride	0.50
Triple Super Phosphate	1.25
Potassium Nitrate	1
Magnesium Sulphate	0.15
Sodium bicarbonate	8.0
Sodium Nitrate	1.25
Cooking Soda	1.5

The combination of Rao's media as

Ingredients	g/l
Calcium Chloride	0.04
Sodium Chloride	0.50
Triple Super Phosphate	1.25
Potassium Nitrate	1
Magnesium Sulphate	0.15
Sodium bicarbonate	8.0
Sodium Nitrate	1.25
Cooking Soda	1.5

In our culture period the described Revised media-6, OFERR media, CFTRI media, Zarruk media, Rao's media and JUST media (Self media) were replicate with three replica. These three replicas are maintained with same physical and chemical factor. Three replicas for Revised media were called as Revised media-1, Revised media-2,Revised media-3, These three replicas

for OFERR media were called as OFERR-1, OFERR-2, OFERR-3, . Three replicas for Revised media are called as CFTRI-1, CFTRI-2, CFTRI-3, Zarruk-1, Zarruk-2, Zarruk-3, Self media (JUST-1), Self media (JUST-2), Self media (JUST-3).

Luminosity

On the culture period light were maintained by Thai-glass of laboratory window. These types of glass prevent heat and only light can access through it. We measure that in day time the light intensity. The light intensity was 530/cm²/mw. On culture period, first 3 days serve 24 hour light facility. After that the photoperiod was 12:12. It show high growth rate of filament.

Temperature

Temperature is a vital factor of grow of *Spirulina maxima*. From the beginning of culture period the room and the water temperature was above 27 to below 36. Some days are very hot but rainy so that temperature fluctuation into two days was not more than 2-3°C. The water temperature was always 1-2°C above the room temperature.

Dissolved solids:

On the culture period media was prepared with given chemical formula and distilled water. On this formula Triple super Phosphate was used to meet phosphorus demand of the species. But it made huge amount of suspended solids.Dissolvedsolids in water were less thansuspended solids on water in the media.

pH:

pH had a direct effect on the physiological properties of algae and the availability of nutrient. pH determined the solubility of carbon source and minerals in the culture directly or indirectly. *Spirulina* grew well at pH values between 9 and 11. The optimal pH of the *Spirulina* nutrient medium was shifted from 8.4 to 9.5 during the mass cultivation, due to the consumption of bicarbonate and sodium ions.

Water quality

The characteristics of water quality contributed in the algal mass production. It had dual influence, firstly by affecting the solubility of nutrients added in the medium and also selective accumulation of certain heavy metals by algae during the growth phase

Working Procedure:

We collected *Spirulina maxima* from BCSIR, Dhaka known as Science laboratory. We collected Spirulina from there and carry it with plastic pots.

Figure 2: In BCSIR culture period.

Before starting aquarium culture, in JUST Fisheries and Marine Bioscience laboratory at first we washed the aquarium with detergent and dry it up. Then we prepared the media with 30 liter distilled water with given chemicals. After twenty four hours we transferred the *Spirulina maxima* at the volume of 500 ml. in each aquarium which consist 30 liter media. After transferring species we facilitate with light for 72 hours continuously.

72 hours after transferring species to culture media we changed the photoperiod. We made it with 12 hours light and 12 hours dark.

From the first day of transferring the species, aeration was done in every aquarium for 24/7.

The culture period was 45 days. From August, 29 to October, 12 was the culture period.

The BCSIR preserved the mother sample in flux, and gave aeration and agitation through aerator. We also preserved the mother sample as BCSIR.

Figure 3: Preserving the Mother spirulina in BCSIR.

Figure 4: Flux culture in Fisheries Lab.

Figure 5: Aquarium Culture in Fisheries Lab.

Monitoring of culture media

Temperature

The culture media was monitored very carefully. In every day temperature was measured by using thermometer. The temperature was 26 to 35°C. The fluctuation between two experimental days was not more than 3°C. In the high temperature day in the culture media species show the best growth rate.

pH

pH was measured by pH meter in every experimental day. The pH varies from 8 to 11 in the total culture period. On the initial time of experiment the pH was very low as 7.5 to 8. But it was increasing with culture time and temperature.

Salinity

We carefully measured salinity with salinity meter. The initial salinity was 7 to 9. But it is increases with culture period. At the final time of experiment the salinity was reached 16 with addition of some chemicals like Urea and Sodium bicarbonate at the volume of 30g in each aquarium along with 5g. Sodium bicarbonate was added only in Self (JUST-1,2,3) media.

Figure 6: Observing the Salinity by salinity meter

Microscopic Condition

We carefully observed the species under microscope at all the culture period every day at two times a day. At first we observed at morning and end of the day in evening. Under microscope we actually observed the health condition of Spirulina filament, its shape, color, growth, optical density. We also measured the optical density by using sewzuic Rafter cell called the SR cell under electronic microscope.

Figure 7: Observing the microscopic condition of Spirulina.

Contamination observation

We used to observe the contamination by human eyes and through microscope. Sometimes we found debris in microscope slide such non dissolvable chemical particles, few dust, and sometimes we found some insects larvae and dead insects in the culture media.

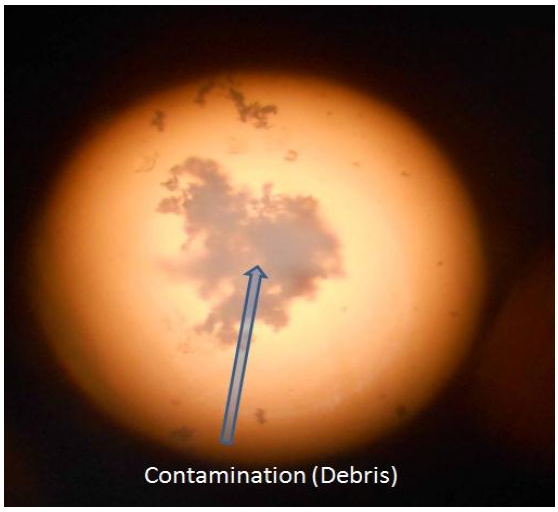

Figure 8: Contamination found in culture period.

Shaking device

In our total culture period we used the rotator as shaking device. The device was used for good mixing of media and species and also prevents the Spirulina layer on the aquarium water surface. The rotator was used in aquarium for 24/7. In every aquarium we used single water rotator.

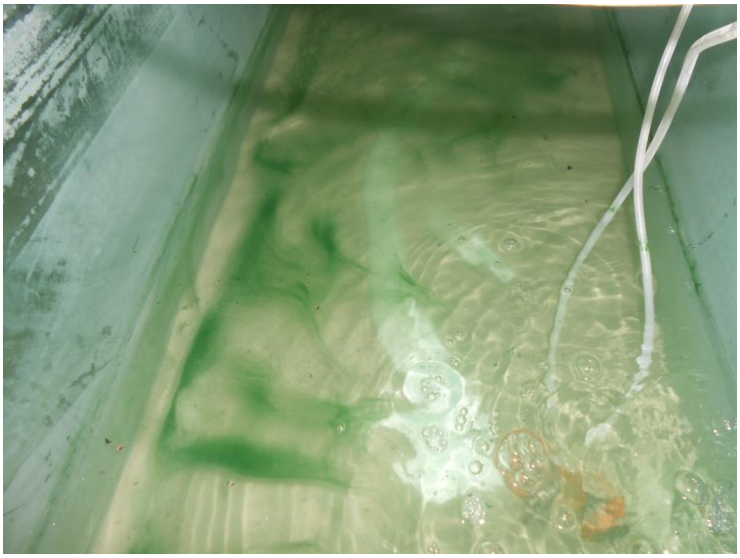

Figure 9: Shaking and Aeration with Aerator.

Aeration devices

Aeration device was used in every aquarium. In each aquarium one aerator was used for aeration to the species for 24/7 in total culture period.

Harvestingand processing

After completing the culture of Spirulina we harvested the species from media. We followed several steps to get complete raw dry Spirulina. These steps were given below

1. Filtration and cleaning:A nylon filter was used to filtration the harvested Spirulina raw sample.
2. Pre-concentration: To obtain algal biomass this is washed to reduce salts content;
3. Concentration: To remove the highest possible amount of interstitial water (located among the filaments);

Figure 10: Harvest Process

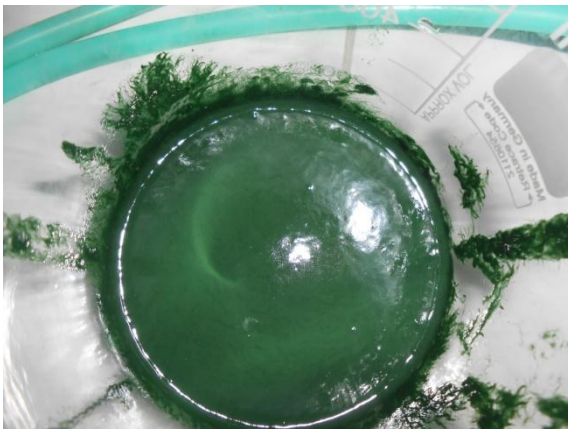

Figure 11: Harvested Spirulina

4. Neutralization: To neutralize the biomass with the addition of acid solution;
5. Dehydration by spray-drying: This operation has great economic importance since it involves about 20–30 percent of the production cost;

Conclusion

Spirulina appears to have considerable potential for development, especially as a small-scale crop for nutritional enhancement, livelihood development and environmental mitigation. In particular, the production and use of spirulina has the following advantages: it provides an easily digestible high (c. 60 percent) protein product with high levels of β-carotene, vitamin B12, iron and trace minerals and the rare essential fatty acid γ-linolenic acid (GLA). In addition, it has no obvious negative cultural or religious issues associated with its consumption; its production occupies only a small environmental footprint, with considerable efficiencies in terms of water

use, land occupation and energy consumption when compared to traditional terrestrial crops; its production is especially suitable to saline and alkaline conditions that are often unfavorable to traditional crops and are frequently occupied by disadvantaged people suffering from, or vulnerable to, natural disasters; its production can be conducted at a number of different scales, from household “pot culture” to intensive commercial development over large areas; it has the potential for integration with rural organic waste treatment processes to improve both environmental conditions and improve energy transfer efficiencies.